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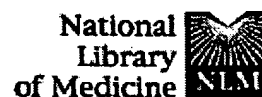
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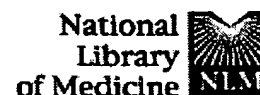
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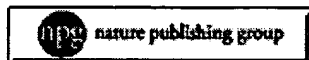
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Combination gene therapy with adenoviral vector-mediated HSV-tk+GCV and IL-12 in an orthotopic mouse model for prostate cancer.

Nasu Y, Bangma CH, Hull GW, Yang G, Wang J, Shimura S, McCurdy MA, Ebara S, Lee HM, Timme TL, Thompson TC.

Scott Department of Urology, Baylor College of Medicine, Houston, Texas, USA.

We previously demonstrated significant therapeutic activities associated with adenoviral vector-mediated Herpes Simplex Virus/thymidine kinase (AdHSV-tk) with ganciclovir (GCV) in situ gene therapy in the RM-1 orthotopic mouse prostate cancer model and interleukin-12 (AdmIL-12) in situ gene therapy in the RM-9 orthotopic mouse prostate model for prostate cancer. In both protocols, local cytotoxicity and activities against pre-established lung metastases were demonstrated. To test whether combined AdHSV-tk+GCV+IL-12 gene therapy would lead to enhanced therapeutic effects when compared to either treatment alone, we used RM-9 mouse prostate cancer cells in both orthotopic and pre-established lung metastases models of prostate cancer. Combined treatment with a single injection of optimal doses of AdHSV-tk+GCV or AdmIL-12 led to significantly increased suppression of orthotopic tumor growth. IL-12 gene therapy alone was more effective than AdHSV-tk+GCV in suppressing spontaneous lymph node metastases and pre-established lung metastases but combination gene therapy did not result in additional anti-metastatic activities. Combination gene therapy also did not achieve significantly better animal survival compared to AdHSV-tk+GCV or AdmIL-12 alone. Analysis of localized antitumor activities demonstrated that AdHSV-tk+GCV therapy induced higher levels of necrosis compared to AdmIL-12 or combination therapy. However, both treatments alone and combination therapy produced similar increases in apoptotic index. To address the possible mechanisms of locally co-operative cytotoxic activities, we analyzed the systemic natural killer (NK) response and the numbers of tumor-infiltrating immune cells using quantitative immunohistochemical analysis. AdHSV-tk+GCV therapy alone led to detectable increases in iNOS-positive cells, CD4+ and CD8+ T-cells and moderately increased numbers of F4/80 (macrophage selective)-positive cells within treated tumors. In contrast, AdmIL-12 elicited a highly robust pattern of tumor infiltration for all four of these immune cells that was in general mimicked by combination therapy. Further analysis of the accumulation of transforming growth factor-beta1 (TGF-beta1) immunohistochemical staining demonstrated that AdHSV-tk+GCV treatment, but not AdmIL-12 treatment, produced cancer cell-associated increases in this cytokine relative to control Ad-beta-gal injections. Interestingly, local injection with AdHSV-tk+GCV induced significant splenocyte-derived NK cell cytolytic activities with maximal response 7 days following treatment, whereas AdmIL-12 injection produced significantly higher NK activity with maximal response 2 days following injection. The combined treatment produced a higher systemic NK response over the 14-day treatment period. Depletion of NK cells in vivo demonstrated that this immunocyte subpopulation was responsible for early locally cytotoxic activities induced by AdHSV-tk+GCV but not AdmIL-12 and that NK activities were largely responsible for activities against pre-established metastases generated by both gene therapy protocols. Prostate Cancer and Prostatic Diseases (2001) 4, 44-55

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Prevention of diabetes in the NOD mouse by intra-muscular injection of recombinant adeno-associated virus containing the preproinsulin II gene.**Jindal RM, Karanam M, Shah R.**Department of Surgery, Indiana University School of Medicine, Indianapolis, USA.
r.jindalr@clinmed.gla.ac.uk

Using the Adeno-associated virus (AAV) as a gene delivery vehicle, we have constructed a recombinant vector containing the full length rat preproinsulin gene (vLP-1). Utilizing the well described non-obese diabetic (NOD) mouse model, an experimental group (n = 10) of animals were intramuscularly (i.m.) injected with 10(7) rAAV virions containing the insulin gene and compared to a mock-injected control group (n = 10). Blood glucose (glc) was then measured weekly for 16 weeks. Data showed that the experimental group contained 70% euglycemic animals (defined as glc < 200 mg/dL) versus 10% of the control animals (P < .05) at 14 weeks. Mean weight in the treated group was greater than the untreated group. Insulin mRNA was detected at the injection site of all of the treated animals, but not controls. Complete destruction of islets was confirmed by histology ruling out the possibility of spontaneous reversal of insulinitis. We conclude that i.m. delivery of the insulin gene in the NOD mouse was able to prevent clinical DM up to 14 weeks in a majority of treated animals. Our experimental data suggests that gene therapy may be an alternative treatment for IDDM in the future.

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Gene therapy for hereditary hematological disorders.

Herzog RW, Hagstrom JN.

Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, USA. rwherzog@mail.med.upenn.edu

The year 2000 saw the first successful treatment of a genetic disorder by gene therapy. Pediatric patients with X-linked severe combined immunodeficiency disorder (SCID-X1) received autologous CD34+ hematopoietic cells following ex vivo gene transfer using a retroviral vector, with subsequent demonstration of improved immune responses. A number of preclinical and clinical studies have been conducted with the aim of developing gene therapy for hemophilia, Fanconi anemia, sickle cell disease, beta-thalassemia, chronic granulomatous disease, and other inherited hematological disorders. The greatest advances in novel approaches toward treatment of hematological disorders have been made in hemophilia, with 3 current phase I clinical trials ongoing. Two trials are investigating the safety and feasibility of utilizing either an ex vivo, non-viral gene transfer technique or an intravenous infusion of a retroviral vector to treat adults with severe hemophilia A (factor VIII deficiency). The third study involves intramuscular administration of an adeno-associated viral (AAV) vector for expression of factor IX in adult patients with hemophilia B. Results from this study and from preclinical studies preceding the trial demonstrate that it is possible to safely administer high doses of a viral vector in vivo.

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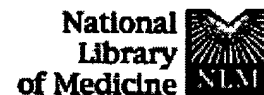
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Department of Physiology, College of Medicine, University of Florida Brain Institute, Gainesville, Florida, USA.

Cardiovascular disease is the leading cause of mortality and morbidity in developed countries. Most conventional therapy is often inefficient and tends to treat the symptoms rather than the underlying causes of the disorder. Gene therapy offers a novel approach for prevention and treatment of cardiovascular diseases. Technical advances in viral vector systems and the development of fusogenic liposome vectors have been crucial to the development of effective gene therapy strategies directed at the vasculature and myocardium in animal models. Gene transfer techniques are being evaluated as potential treatment alternatives for both genetic (familial hypercholesterolemia) and acquired occlusive vascular diseases (atherosclerosis, restenosis, arterial thrombosis) as well as for cardiac disorders including heart failure, myocardial ischemia, graft coronary arteriosclerosis and hypertension. Continued technologic advances in vector systems and promising results in human and animal gene transfer studies make the use of gene therapy a promising strategy for the treatment of cardiovascular disorders.

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**Application of conditionally replicating herpes vector for gene therapy treatment of urologic neoplasms.****Oyama M, Yazaki T, Ohigashi T, Hoshi M, Horiguchi Y, Oya M, Asakura H, Nakashima J, Tachibana M, Uyemura K, Murai M.**Department of Urology, School of Medicine, Keio University, Tokyo, Japan.
moyama@med.keio.ac.jp

Herpes vector has been widely used for experimental gene therapy. We herein review the strategies of such therapy for the treatment of urologic neoplasms. Most experimental studies of genetically altered viruses have employed replication-incompetent vectors. However, such viruses are unable to infect additional cells subsequent to the initial infection event. Therefore, this strategy has relied heavily on the bystander effect because a large number of noninfected tumor cells remain. Conditionally replicating herpes vector G207 has been developed in order to overcome potential problems of safety and tumor specificity for human use. It has been used to treat malignant brain tumors because of its neural tropism. In the last few years, applications of G207 for non-neural tumors have been reported. Because G207 may be useful for the treatment of urologic malignant tumors, we evaluated the antitumor effect against several types of tumor cells both in vitro and in vivo. Our data suggest that G207 may be applicable for the treatment of urologic malignant tumors.

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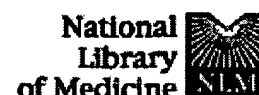
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Tissue-specific promoters in gene therapy for the treatment of prostate cancer.

Shirakawa T, Gotoh A, Wada Y, Kamidono S, Ko SC, Kao C, Gardner TA, Chung LW.

Department of Urology, Kobe University School of Medicine, Kobe, Japan. toshiro@kobe-u.ac.jp

Delivery of therapeutic toxic genes to and their expression in tumor cells through the use of tissue-specific promoters could decrease their toxic effect on neighboring normal cells when virus-mediated gene delivery results in their infection. We have demonstrated the utility of two prostate cancer-specific promoters, long PSA and osteocalcin, for tissue-specific toxic gene therapy for prostate cancer. The two promoters were highly active in both androgen-dependent and androgen-independent prostate cancer cells. We also introduce the Phase I trial of osteocalcin promoter-based toxic gene therapy for bone metastases of prostate cancer, which is in progress at the University of Virginia.

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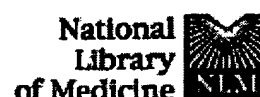
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Hammerhead ribozymes as therapeutic agents for bladder cancer.**Irie A, Kashani-Sabet M, Scanlon KJ, Uchida T, Baba S.**Department of Urology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan.
akira_irie@pop07.odn.ne.jp

Hammerhead ribozymes have been investigated extensively as therapeutic agents against cancer. Aberrant or overexpression of genes related to tumorigenicity or cancer growth might be the appropriate targets for ribozyme strategies. Ribozyme-mediated gene therapy should be applied to those diseases that have no successful conventional therapy such as advanced or treatment-resistant bladder cancer. Many genetic alterations have been identified in bladder cancer related to both tumorigenesis and disease progression. Mutated H-ras, fos, and erb-B2 genes have been chosen as targets for ribozymes in previous studies, and antitumor efficacy has been demonstrated by reversion of the malignant phenotypes and by inhibition of tumor growth both in vitro and in vivo. The efficiency of various delivery systems has also been evaluated. An overview of ribozyme strategies, especially for therapeutic applications against bladder cancer, is described here.

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**Ex vivo gene therapy using granulocyte-macrophage colony-stimulating factor-transduced tumor vaccines.****Kawai K, Tani K, Asano S, Akaza H.**

Department of Urology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan. rkawa@md.tsukuba.ac.jp

There is no standard effective therapy for metastatic renal-cell carcinoma (RCC) or prostate cancer. Both of these cancers may be immunogenic, so therapy targeted to a tumor-associated antigen may be effective. Transduction of the gene encoding granulocyte-macrophage colony-stimulating factor has shown promise in preclinical studies, and clinical trials are in their early stages. Both autologous cancer cells and partially HLA-matched allogeneic cells are being studied. No dose-limiting side effects have been observed, and a few patients have had transient objective tumor regressions. Further trials with more frequent and, probably, longer immunization schedules are needed to define efficacy.

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**Combined delivery of an antiangiogenic protein (angiostatin) and an immunomodulatory gene (interleukin-12) in the treatment of murine cancer.**

Wilczynska U, Kucharska A, Szary J, Szala S.

Department of Molecular Biology, Center of Oncology Maria Skłodowska-Curie Memorial Institute, Gliwice, Poland.

We investigated the feasibility of a novel therapeutic approach to treat neoplastic diseases in mice. This novel strategy consists in delivering a protein (angiostatin) with strong antiangiogenic properties, followed by administration of the interleukin 12 gene that is strongly immunomodulatory and has also some antiangiogenic effects. When angiostatin-mediated antiangiogenic therapy was used in combination with intratumor delivery of the IL-12 gene (a strategy much safer than IL-12 protein administration), this produced a synergistic therapeutic effect.

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